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From the INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY <del>S Received</del>

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Date:

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY **EXAMINATION REPORT** 

F/E				(PCT Rule 71.1)	
			Date of mailing (day/month/year)	23.07.2004	
Applicant's or agent's file reference P32590WO/NCB			імро	PRTANT NOTIFICATION	
International application No. PCT/GB 03/03192	International fili 25.07.2003		oketing	Priority date (day/month/year) 26.07.2002	
Applicant ROSLIN INSTITUTE (EDINBURGH) et al.		NOT			
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- The applicant is hereby notified that this International Preliminary amining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
- A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
- Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

#### REMINDER

To:

BASSIL, Nicholas Charles **KILBURN & STRODE** 

20 Red Lion Street

London WC1R 4PJ

**GRANDE BRETAGNE** 

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:

European Patent Office D-80298 Munich

Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465

Tel. +49 89 2399-2926

Brandt, M

**Authorized Officer** 



## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

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	icant's 2590V	_	ent's file reference CB	FOR FURTHER A	CTION		on of Transmittal of Internati camination Report (Form PC	
1	nationa T/GB		ication No. 3192	International filing date 25.07.2003	(day/mon	th/year)	Priority date (day/month/) 26.07.2002	year)
C12	International Patent Classification (IPC) or both national classification and IPC C12N15/62  Applicant  Applicant							
	icant SLIN	INST	TITUTE (EDINBURGH	) et al.			WIPO PO!	
1.	<ol> <li>This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</li> </ol>							
2.	2. This REPORT consists of a total of 5 sheets, including this cover sheet.							
	This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).							
	These annexes consist of a total of 4 sheets.							
3.	This	repo	rt contains indications re	lating to the following if	tems:			
	1	$\boxtimes$	Basis of the opinion					
	II		Priority					
	Ш		•	opinion with regard to r	ovelty, i	nventive step a	and industrial applicabilit	<b>v</b>
	IV		Lack of unity of inventi	•	•	•	• •	•
	V  Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement				l applicability;			
	VI		Certain documents cite	ed				
	VII			international applicatior				
	VIII		Certain observations o	on the international app	lication			
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# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/GB 03/03192

<ol> <li>Basis of the report</li> </ol>	I.	<b>Basis</b>	of th	e report
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1. With regard to the **elements** of the international application (Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)):

	De	escription, Pages			
	1-4	49	as originally filed		
	CI	aims, Numbers			
	1-	15	received on 08.07.2004 with letter of 07.07.2004		
	Dr	awings, Sheets			
	1/2	1-21/21	as originally filed		
2	2. With regard to the <b>language</b> , all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.				
	The	ese elements were a	vailable or furnished to this Authority in the following language: , which is:		
		the language of a t	ranslation furnished for the purposes of the international search (under Rule 23.1(b)).		
		the language of pu	blication of the international application (under Rule 48.3(b)).		
		the language of a tage Rule 55.2 and/or 55	ranslation furnished for the purposes of interpolitical and in the contract of the purposes of interpolitical and in the contract of the purposes of interpolitical and in the contract of the purposes of interpolitical and in the contract of the purposes of interpolitical and in the contract of the purposes of interpolitical and in the contract of the purposes of interpolitical and in the contract of the purposes of interpolitical and in the contract of the purposes of the contract of the c		
3.	Wit inte	h regard to any <b>nucl</b> rnational preliminary	eotide and/or amino acid sequence disclosed in the international application, the vexamination was carried out on the basis of the sequence listing:		
			ernational application in written form.		
		filed together with the	ne international application in computer readable form.		
		furnished subseque	ently to this Authority in written form.		
		furnished subseque	ently to this Authority in computer readable form.		
		The statement that	the subsequently furnished written sequence listing does not go beyond the disclosure application as filed has been furnished.		
		The statement that listing has been furn	the information recorded in computer readable form is identical to the written sequence nished.		
4.	The	amendments have i	resulted in the cancellation of:		
		the description,	pages:		
		the claims,	Nos.:		
		the drawings,	sheets:		

### INTERNATIONAL PRELIMINARY **EXAMINATION REPORT**

International application No.

PCT/GB 03/03192

5. 🗆	This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

- 6. Additional observations, if necessary:
- V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- 1. Statement

Novelty (N)

Yes: Claims

1-15

No:

Claims

Yes: Claims Claims

1-15

Industrial applicability (IA)

Inventive step (IS)

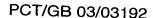
No:

Yes: Claims

No: Claims 1-15 (?)

2. Citations and explanations

see separate sheet



## Additional remarks to section V:

#### 1. Novelty (Article 33(2) PCT)

- The present application discloses the use of a nucleic acid construct, comprising a 1.1 sequence encoding a lipocalin, for the detection of a gene activation event resulting from a change in a cell. Said change may be toxicological stress, a metabolic change or a disease.
- The documents mentioned in this communication are numbered as in the International Search Report (ISR), i.e. D1 corresponds to the first document of the ISR etc.
- 1.3 The present application satisfies the criterion set forth in Article 33(2) PCT because the subject matter of claims 1-15 is novel in view of the cited documents.

#### Inventive step (Article 33(3) PCT) 2.

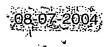
It seems that none of the cited prior art documents discloses the use of a member of the lipocalin protein family as a reporter gene. Lipocalins are know in the art, their fusion with epitopes is known (e.g. in D1 or D7) but none of the documents suggests their usefulness as a reporter gene. Therefore it seems that the use of a lipocalin as a reporter gene, or a method of detecting gene activation using a lipocalin reporter construct, can be considered inventive (claims 1-15).

#### Industrial applicability (Article 33(4) PCT) 3.

The subject matter of claims 1-11 relates to a use for detection of a gene activation event in vitro or in vivo. Said gene activation event can be a disease. Thus the claims encompass a method of diagnosis performed in vivo on the human or animal body. The subject matter of claims 12-15 encompasses a method of diagnosis of a disease performed on a non-human animal. Thus the subject matter of claims 1-15 includes methods of diagnosis of the human or animal body and is thus excluded from examination by Article 34(4)(a)(I) PCT in combination with Rule 67(iv) PCT. For the assessment of these claims on the question whether they are industrially applicable, no unified criteria exist in PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject matter

## INTERNATIONAL PRELIMINARY International application No. PCT/GB 03/03192 EXAMINATION REPORT - SEPARATE SHEET

of claims to the use of a compound in medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment. The applicant is already informed that in the case of a European application, claims 1-15 do not seem to be allowable because 'diagnostic methods practised on the human or animal body shall not be regarded as inventions which are susceptible of industrial application'.



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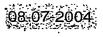
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### **CLAIMS**

- 1. The use of a nucleic acid construct comprising a nucleic acid sequence encoding a member of the lipocalin protein family as a reporter gene for the detection of a gene activation event resulting from a change in or altered metabolic status in a cell in vitro or in vivo, wherein said cell is transfected with said construct.
- 2. The use as claimed in claim 1, in which the lipocalin protein is heterologous to the cell in which it is expressed.
- 3. A use as claimed in claim 1, in which the lipocalin protein is coded for by a nucleic acid construct comprising (i) a nucleic acid sequence encoding a member of the lipocalin protein family, and (ii) a nucleic acid sequence encoding a peptide sequence of from 5 to 250 amino acid residues
- 4. A use as claimed any one of claims 1 to 3, in which the lipocalin is selected from the group consisting of: ovine betalactoglobulin (BLG) (accession No. X12817), murine major urinary protein (MUP) (accession No. NM 031188) and rat  $\alpha$ -2-urinary globulin ( $\alpha$ -2u) (accession number M27434).
- 5. A use as claimed in claim 3 or claim 4, in which peptide sequence is an epitope.
- 6. A use as claimed in claim 5, in which the epitope is selected from the group consisting of EQKLISEEDL, GKPIPNPLLGLDST, YPYDVPDYA, NVRFSTIVRRRA, KQMSDRRENDMSPS, SGNEVSRAVLLPQSC, SSLSYTNPAVAATSANL, RSTLQHPDYLQEYST, VSTLLRWERFPGHRQA, KFQQLVQCLTEFHAALGAYV, QEQCQEVWRKRVISAFLKSP, and RLSDKTGPVAQEKS

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7. A use as claimed in any one of claims 2 to 6, in which the construct additionally comprises a promoter element upstream of the (i) a nucleic acid sequence encoding a member of the lipocalin protein family, and (ii) and nucleic acid sequence

encoding a peptide sequence of from 5 to 250 amino acid residues.

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8. A use as claimed in claim 7, in which the promoter element may be selected from one of the following groups consisting of:

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(i) c-myc, p21/WAF-1, MDM2, Gadd45, FasL, GAHSP40, TRAIL-R2/DR5, BTG2/PC3;

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(ii) MnSOD, CuZnSOD, IkB, ATF4, xanthine oxidase, COX2, iNOS, Ets-2, FasL/CD95L, yGCS, ORP150.

(iii) Lrg-21, SOCS-2, SOCS-3, PAI-1, GBP28/adiponectin, α-1 acid

glycoprotein, metallothioneine I, metallothioneine II, ATF3, IGFbp-3, VDGF and HIF1 a.

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(iv) Gadd 34, GAHSP40, TRAIL-R2/DR5, c-fos, CHOP/Gadd153, APAF-1, Gadd45, BTG2/PC3, Peg3/Pwl, Siah1a, S29 ribosomal protein, FasL/CD95L, tissue tranglutaminase, GRP78, Nur77/NGFI-B, CyclophilinD, p73 and Bak.

(v) a promoter from a xenobiotic metabolising cytochrome p450 enzymes from the 2A, 2B, 2C, 2D, 2E, 2S, 3A, 4A and 4B gene families.

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(vi) a synthetic promoter sequence comprised of a minimal eukaryote consensus promoter operatively linked to one or more response elements selected from the group consisting of the aryl hydrocarbon (Ah)/Ah nuclear translocator (ARNT) receptor response element, the antioxidant response element (ARE), the xenobiotic response element (XRE).

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- 9. A use as claimed in claim 1, in which the nucleic acid construct comprises a stress inducible promoter operatively isolated from a nucleic acid sequence encoding a member of the lipocalin protein family by a nucleotide sequence flanked by nucleic acid sequences recognised by a site specific recombinase, or by insertion such that it is inverted with respect to the transcription unit encoding a member of the lipocalin protein family, in which the construct additionally comprises a nucleic acid sequence comprising a tissue specific promoter operatively linked to a gene encoding the coding sequence for the site specific recombinase.
- 10. A use as claimed in claim 9, in which the site specific recombinase sequences are two loxP sites of bacteriophage P1.
  - 11. A use as claimed in any one of claims 1 to 10, in which the gene activation event is induction of toxicological stress, metabolic changes, or disease, including a disease that is the result of viral, bacterial, fungal or parasitic infection.
  - 12. A method of detecting a gene activation event in a cell in vitro or in vivo, comprising assaying a host cell stably transfected with a nucleic acid construct comprising a nucleic acid sequence encoding a member of the lipocalin protein family, or a transgenic non-human animal whose cells express such a construct, in which the cell or animal is subjected to a gene activation event that is signalled by expression of a peptide tagged lipocalin reporter gene.
  - 13. A method of screening for, or monitoring of toxicologically induced stress in a cell or a cell line or a non-human animal, comprising the use of a cell, cell line or non human animal which has been transfected with or carries a nucleic acid construct as defined in any one of claims 2 to 10.
- 14. A method for screening and characterising viral, bacterial, fungal, and parasitic infection comprising the use of a cell, cell line or non human animal which has been



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transfected with or carries a nucleic acid construct as defined any one of claims 2 to 10.

15. A method for screening for cancer, inflammatory disease, cardiovascular disease, metabolic disease, neurological disease and disease with a genetic basis comprising the use of a cell, cell line or non human animal which has been transfected with or carries a nucleic acid construct as defined in any one of claims 2 to 10.